






# Taxonomic report of filamentous fungi presents in sewage waste sludge with biotechnological potential

## Reporte taxonómico de hongos filamentosos presentes en lodo residual de depuradora con potencial biotecnológico

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**Abstract** There is few information regarding the identification of filamentous fungi present in sewage sludge, the importance of these microorganisms lies in the potential use they have in the food, pharmaceutical and agri–environmental industries as they are beneficial in the remediation of water, sludge and soil contaminated by Heavy metals and hydrocarbons also contribute to the adsorption of nutrients by crops. The use of residual sludge once decontaminated by these microorganisms can be used in agriculture as soil structure improvers, organic fertilizers and as an amendment to prevent soil erosion. The objective of this research was to characterize and identify fast and medium–growing filamentous fungi from sewage sludge. The residual sludge was collected directly from the thickness of the municipal water treatment plant of Cd. Victoria, Tamaulipas, Mexico. Subsequently, the fungi were isolated on agar plates and identified using morphological characters of the colonies and by PCR and diana sequences. The species identified were: *Trichoderma longibrachiatum*, *Aspergillus terreus*, *Tylophilus porphyrosporus* and *Aspergillus fumigatus*. Being the first report for Mexico of fungal species that grow in residual sludge from water treatment plants. The molecular characterization of the species of filamentous fungi found in sewage sludge will allow progress in research focused on knowing the advantages and biological and chemical mechanisms of these microorganisms for the development of biotechnological processes and products, with high agricultural potential and environmental.

**Keywords:** Genus, molecular identification, morphology, species.

**Resumen** Existe poca información respecto a la identificación de hongos filamentosos presentes en lodos de depuradora, la importancia de estos microorganismos radica en el uso potencial que tienen en la industria alimentaria, farmacéutica y agroambiental al ser benéficos en la remediación de agua, lodos y suelo contaminados por metales pesados e hidrocarburos, además, de contribuir en la adsorción de nutrientes por los cultivos. El uso de lodos residuales una vez descontaminados por estos microorganismos puede ser utilizados en la agricultura como mejoradores de la estructura del suelo, abonos orgánicos y como enmienda para evitar la erosión del suelo. El objetivo de esta investigación fue caracterizar e identificar los hongos filamentosos de rápido y mediano crecimiento a partir de lodos de depuradora. El lodo residual se recolectó directamente del espesor de la planta potabilizadora municipal de Cd. Victoria, Tamaulipas, México. Posteriormente se realizó el aislamiento de los hongos en placas de agar y se identificaron empleando caracteres morfológicos de las colonias y mediante PCR y secuencias diana. Las especies identificadas fueron *Trichoderma longibrachiatum*, *Aspergillus terreus*, *Tylophilus porphyrosporus* y *Aspergillus fumigatus*. Siendo este el primer reporte para México de especies fúngicas que crecen en lodos residuales de plantas potabilizadoras. La caracterización molecular de las especies de los hongos filamentosos encontradas en los lodos de depuradora permitirá avanzar en investigaciones enfocadas en conocer las ventajas y mecanismos biológicos, químicos de estos microorganismos para el desarrollo de procesos y productos biotecnológicos, con alto potencial agrícola y ambiental.

**Palabras clave:** Especie, género, identificación molecular, morfología.

## Introduction

Filamentous fungi (Ff) have a wide distribution in the world, they are eukaryotic organisms, with filamentous structures, called hyphae, they lack chlorophyll, they can be aerobic or facultative anaerobes and have sexual and asexual reproduction. Are used in the field of environmental and industrial biotechnology due to their ability to produce various compounds such as: antibiotics, meat products and for the removal of potentially toxic elements (PTEs), which are useful in the pharmaceutical, food and environmental fields, respectively (Chu *et al.*, 2021; Suárez-Contreras & Peñaranda-Figueroa, 2022).

Currently, there is few information within bioremediation where the fungal mass of waste sludge from municipal plants is used as a potentially efficient and PTEs tolerant raw material for removal (Bonito *et al.*, 2010).

Filamentous fungi contribute to the degradation and mineralization of organic matter through physiological and biochemical mechanisms (Oshiquiri *et al.*, 2020; Sun *et al.*, 2020; Manna *et al.*, 2020). In addition, they absorb PTEs and essential components that can be used in biotechnological procedures for agricultural and environmental purposes. Having the ability to adsorb PTEs through mycelium, several species of filamentous fungi such as: genres *Trichoderma* and *Aspergillus*, have been described and characterized revealing their potential to be employed as PTEs biosorbents (Cadavid-Velásquez *et al.*, 2019), considering the degree of tolerance, which is different for each species and for PTEs (Vallejo *et al.*, 2021).

In this sense, the generation of knowledge on the characterization and identification of Ff, both at the microscopic, macroscopic, and molecular level, is decisive to differentiate species and select the most favorable ones

in remediation processes of contaminated sites. It should be noted that characterizing filamentous fungi is vital since apart from their environmental use, their potential has also been reported, as biological controllers, they participate in the degradation of complex organic matter, converting it to simple chemical forms that become part of the soil to that can be absorbed by plants, contributing to soil fertility (Chu *et al.*, 2021).

Studies on the characterization of Ff genera and species during the composting process of municipal waste sludge (MRL) have not been fully developed in Mexico as a biotechnological potential, mainly to the little importance given to the use of the residual sludge once decontaminated as improvers and organic fertilizers in agricultural soils.

Due to the use of these microorganisms and their reported high biotechnological potential, it is necessary to search for improvements in their isolation and identification processes through different techniques, such as molecular techniques, which are important due to their reliability and practicality to amplify DNA segments assisted by internal transcribed spacers (ITS), a fragment of interest for taxonomic studies (Suárez-Contreras & Peñaranda-Figueroa, 2022). Therefore, the aim of this work was to isolate, characterise and molecularly identify those filamentous fungi present in sludge from wastewater treatment.

## Materials and methods

A protocol is proposed to identify filamentous fungi from samples taken from waste sludge.

### Sampling site

The residual sludge was collected directly from the thickness of the sludge at the Cd. Victoria, Tamaulipas wastewater treatment plant. The analysis of the residual sludge was carried out

in the Microbial Biotechnology laboratory of the Institute of Applied Chemistry (IQA-UV) of the University Veracruzana in Xalapa, Veracruz and in the Applied Ecology laboratory of the Autonomous University of Tamaulipas (IEA-UAT), in Mexico.

### Isolation of fungi from residual sludge from the treatment plant

The isolation of microorganisms, specifically fungus, was carried out from a plate count to isolate the colonies and differentiate the yeast-like and filamentous development of each Colony Forming Unit (CFU). Subsequently, the morphological and molecular characterization of the isolated fungi was carried out. For the isolation of the fungus, six samples were taken from the sludge digester, each sample (10 g) was placed in glass flasks with 90 mL of sterile distilled water (10<sup>-1</sup> dilution). From this dilution, subsequent dilutions were made up to 10<sup>-5</sup>, of which 0.1 mL aliquots were taken and inoculated in Petri dishes with potato dextrose agar (PDA, BIOXON and DIBICO, Mexico). The incubation time was 5 to 10 days at a temperature of 30 °C. Subsequent to the dilutions where the filamentous fungi developed, the colonies were taken and replanted for purification again in PDA medium, and with this, microcultures were carried out.

### Morphological characterization of the strains

The identification of the isolated fungi was based on morphological characteristics of the colonies (color, hyaline or pigmented appearance, consistency, observation of the front and back of the colony, and presence or absence of pigments and exudates), and on microscopic observation (microscope). Binocular 2500x Quasar Qm 18 with Unico brand Usb eyepiece) of microcultures of the fungal structures (presence or not of septa, pigmentation or not of the hyphae, and observation of asexual reproduction structures) to characterize the isolated genera of the

compendium of soil fungi the Domsch *et al.* (2007); Abarca (2000); Arenas (2011); Stephen *et al.* (1997); Valencia & Castro (2004).

### Molecular identification of fungal strains isolated from residual sludge

The extraction of the nine strains was done in duplicate. For the extraction, the REDEXtract-N-Amp Plant kit (Sigma-Aldrich, St. Louis, MO, USA) was used. For this, 1mm<sup>2</sup> of mycelium was placed in 20 µL of ES solution, then processed in the thermocycler with the following program: 65 °C for 10 min, followed by 10 min at 95 °C, and 4 °C to finish. Finally, the samples were diluted in 20 µL of DS solution and allowed to incubate for 30 min at room temperature and then stored at 4 °C (Irinnyi *et al.*, 2015).

The PCR reaction was performed with the primers ITS1F and ITS4 (Gardes & Bruns, 1993) for the 9 samples and their replicates according to the protocol of Garibay-Orijel *et al.* (2013). The MyTaq™ DNA Polymerase PCR kit (Bioline) was used for the reaction. Thermocycler Program: 95 °C for 1 min, followed by 35 cycles at 95 °C, 51 °C and 72 °C for 1 min. Each temperature, and a final extension of 72 °C for 8 min. PCR products were reviewed on a 1 % agarose gel in TBE buffer at 90 volts for 50 min.

### Analysis of DNA

Sequences from both directions of each strain were edited in Geneious 7 (Biomatters). The edited sequences were assembled to 100 % genetic similarity generating consensus sequences. The sequences of all the strains were compared with each other by assembling at 100 % nucleotide similarity "Contigs" with the strains of each species. The consensus sequences of all the Contigs (species) were compared against the GenBank database, by means of BLAST analysis. Additionally, the sequences corresponding to the genera *Aspergillus* and *Trichoderma* were compared against the "ISHAM ITS DATABASE"

database of the "International Society for Human and Animal Mycology ITS Database" (Kõljalg *et al.*, 2005). The sequence that corresponded to *Tylopilus* was also compared against the "UNITE" database.

## Phylogenetic representation

The taxonomic identification of the strains was carried out through phylogenetic analysis. For this, they downloaded from Genbank and ISHAM the sequences related to those of the strains. The alignments were performed in Muscle, to perform Bayesian analyzes with the GTR substitution model with four Monte Carlo chains over 1.000.000 generations, sampling every 400 generations with a temperature value of 0.2, discarding the first 10 % of the generated trees. The support of the branches was calculated by means of their posterior probability (Bpp). In the case of *Trichoderma*, the analysis was carried out specifically for the Longibrachiatum section using *T. viride* and *T. lixi* as external groups (Mata *et al.*, 2016).

## Results and discussion

Nine strains of filamentous fungi were isolated, from which four species were identified:

### *Aspergillus terreus*

**Macroscopic characteristics:** *A. terreus* colonies cultivated in PDA grew from 7 to 10 days, reaching 3 to 3.5 cm in diameter, presenting a white coloration; on the back of the box, a change in the culture medium was observed, from transparent to yellow (**Figure 1a**). At 20 days, the white colonies presented a brown-brown coloration (**Figure 1b**).

**Microscopic features:** The conidial heads are compact biseriate columns, the conidiophores are smooth and hyaline. The vesicles had a variable, columnar shape (subglobose or ellipsoidal, **Figure 1c** and spherical (**Figure 1d**).

The macroscopic and microscopic characteristics were similar to those reported by Abarca (2000), who points out that the colonies appear brown with beveled conidia and subglobose vesicles and of the compendium of soil fungi the Domsch *et al.* (2007). *A. terreus* was identified in the IQA - UV, radial growth was lower at a relative humidity between 60 – 70 %, and at a temperature of 21.7 – 25.5 °C, while at a temperature of 30 °C and a relative humidity close to 80 %, a greater radial growth was obtained with greater aerial mycelium in the IEA - UAT.

### *Aspergillus fumigatus*

**Macroscopic characteristics:** *A. fumigatus* colonies grown on PDA agar grew from 7 days to 10 days at a controlled temperature of 30 °C. Reaching a diameter of 5 to 8 cm presenting a grayish coloration with a white aerial mycelium, no change in the culture medium was observed on the back of the box. After 10 days, the mycelium appeared darker (aging of the colony).

**Microscopic characteristics:** The uniseriate and predominantly columnar conidial heads were distinguished, with hyaline and smooth stems; clearly pyriform or spoon-shaped vesicle. The phialides occupied half or two thirds of the gallbladder. Globose to ovoid conidia, where its unicellular and columnar conidial head was recognized. These characteristics had already been reported from the compendium of soil fungi by Domsch *et al.* (2007).

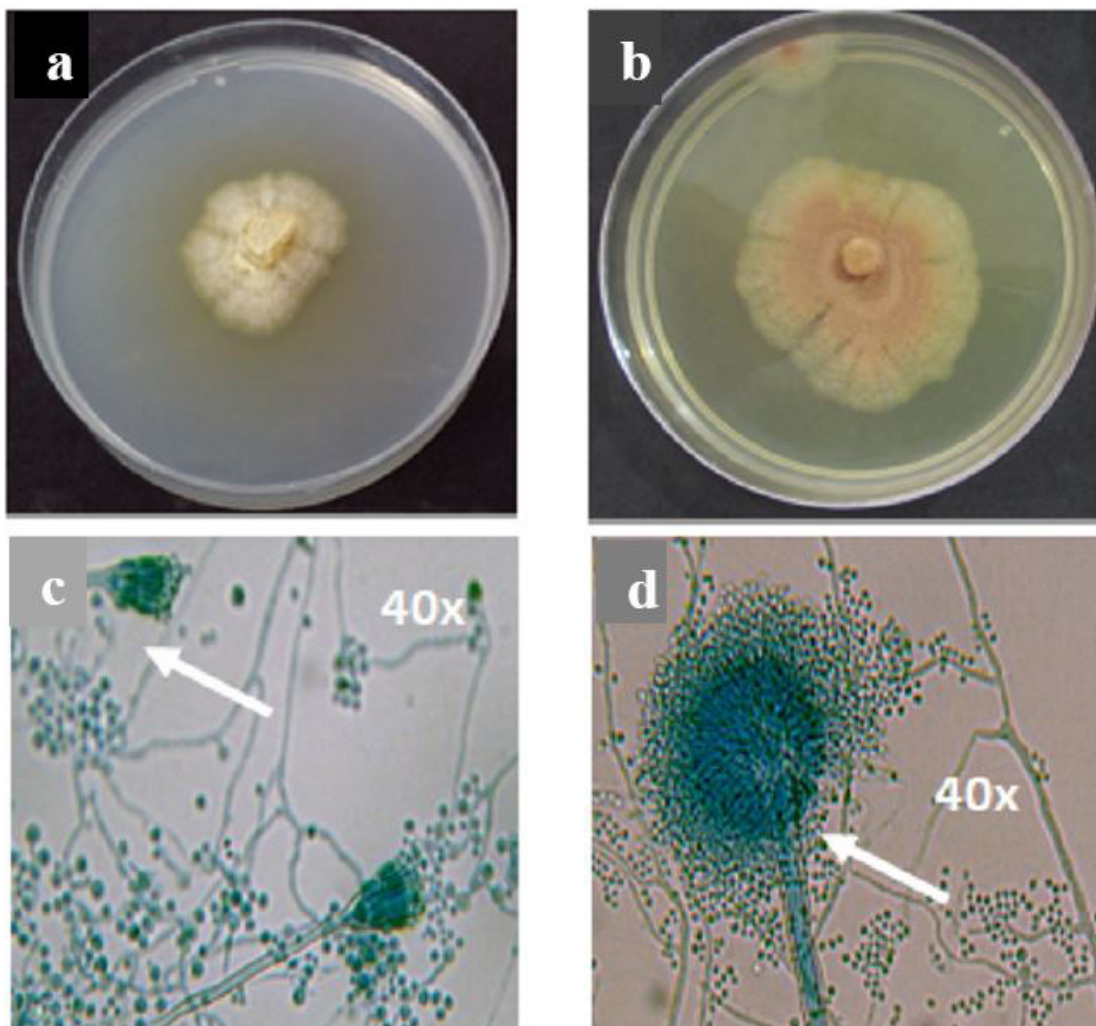
The genus *Aspergillus* was isolated and identified in both laboratories, however, two different species were found, *A. fumigatus* and *A. terreus*. Macroscopically, these two species presented a very different morphology, both in pigmentation and in the growth rate of the colonies, but in microscopic morphology they have a similarity in their conidial heads; however, one is biseriate and with a spherical vesicle (*A. terreus*), and the other is uniseriate

with pear-shaped vesicle (*A. fumigatus*), this morphology allowed to identify its genus, the PCR corroborated genus and species. The reported characteristics are identical to those described by Abarca (2000), who reviewed the

taxonomy of the genus *Aspergillus* and described the characteristics of the species, as did Arenas (2011), Piontelli (2008), and Salazar & Rua (2012) who also report *Aspergillus* morphology like those described in this study.

**Figure 1**

Macroscopic characteristics *A. terreus* in PDA medium at 7 days and 20 days. **1a** and **b** microscopic characteristics at 40x. *A. terreus* Columnar vesicle (subglobose or ellipsoidal), **c** and **d** (globose vesicle)



### ***Trichoderma longibrachiatum***

**Macroscopic characteristics:** *T. longibrachiatum* colonies grown on PDA agar grew from 5 days to 10 days at 25 – 28 °C. Reaching a diameter of 3 to 5 cm, presenting a white and cottony coloration, on the back of the box a change in the culture

medium was observed, from transparent to opaque yellow.

**Microscopic features:** Septate hyphae and branching conidiophores of varying length, some short and long-length conidiophores

extending from the hyphae. The conidia are hyaline and are produced singly at the apex of the conidiophore or directly on the hyphae. Conidia are unicellular, round to pyriform (teardrop-shaped), both conidiophores and hyphae are hyaline.

The factors that determined the diametrical growth of *T. longibrachiatum* in both laboratories (IEA-IQA) were temperature and humidity, as indicated by Guigón-López *et al.* (2010) who found that the growth temperature of *Trichoderma spp.*, oscillates between 27 and 30 °C, simultaneously also carried out a study with microcalorimetric techniques where they determined that the optimum temperature for this genus is 30 °C, although they have the ability to grow at 35 °C. While Guillén-Navarro *et al.* (1998) and Martínez *et al.* (2013), mention that the simultaneity between the temperature and the growth of *Trichoderma* depends on the species and the isolation itself.

*Trichoderma longibrachiatum* was isolated in the two laboratories (IQA-UV and IEA-UAT), however, it presented very different macroscopic characteristics. This is due to the cultivation conditions regarding humidity, light and temperature that were not controlled in the IQA-UV, having a relative humidity of 60 – 70 %, with a temperature of 21.7 – 25.5 °C and approximately 4-5 h light daily. A white mycelium was observed, together with its unicellular and round conidia, which according to the literature indicated belonging to the genus *Absidia*; however, PCR determination identified it as *T. longibrachiatum*. While, in the IEA, the growth of *T. longibrachiatum* was carried out under controlled conditions at 30 °C with a relative humidity of 10 % and exposures of 7 h light, sporulation with greenish tones was observed, giving the appearance of rings in concentric shape.

The results obtained in the IEA-UAT laboratory show a higher diametrical growth

rate at temperatures of 30 °C, which agrees with the results of Valencia & Castro (2004), who isolated *Trichoderma* strains with a higher average diametrical growth rate. At temperatures of 26 and 30 °C. Which has a proportional correspondence with the lower diametrical growth at temperatures below 30 °C as it was in the IQA-UV strain (21.7 °C). Another factor that influenced was the relative humidity, since at a lower humidity, greater growth was observed. Michel-Aceves *et al.* (2001), reported that the number of colony-forming units of *T. longibrachiatum* are higher in places where the relative humidity is lower.

Another morphological difference that was observed in *T. longibrachiatum* was the color of the mycelium, caused by the light intensity. In IQA-UV the light was very limited giving a white and cottony color, while in the IEA-UAT the light was very abundant, and the color Druzhinina of the mycelium was uniform green, both shades of *T. longibrachiatum* coincide with what was reported by Martínez *et al.* (2013). Authors such as Chávez-García *et al.* (2008) and Windham *et al.* (1986) mention that *Trichoderma spp.* is photosensitive, and that it behaves better in daylight conditions; where there is high production of conidia (conidiogenesis) when incubating strains at 25 °C and exposed to light.

Druzhinina *et al.* (2005), point out that the genus *Trichoderma* has a variability among its morphological characteristics, which makes its classification difficult, therefore, molecular techniques are of great relevance. Some species of the genus *Trichoderma* have been reported by Pérez-Sánchez *et al.* (2014) in biosolids from wastewater treatment plants in Puerto Rico. In Mexico, the Ministry of Environment and Natural Resources has reported the presence of filamentous fungi of the genus *Trichoderma* in NOM-004-SEMARNAT-2002. In this study, the first report of *T. longibrachiatum* is made, found in residual sludge from wastewater treatment plants.

## *Tylopilus porphyrosporus*

**Gross features:** No definite colony was observed; however, at 3 days, the color of the agar changed from transparent to opaque white. At 10 days the colony presented a small dark pigmentation in the center of the box.

**Microscopic characteristics:** Unsegmented hyphae were observed, and the presence of spores was not detected in the 10 days of culture, only hyaline hyphae were observed.

## Molecular identification of fungal strains isolated from sewage sludge

When performing the first PCR in samples of mycelium, it was possible to amplify the band corresponding to the general PCR amplified in a 1:1 concentration. Of the nine strains, 88 % amplified, while strains 2 and 8 did not amplify, these two strains were tested with dilutions of the DNA extraction, 1:10, 1:100 and 1:1000 with 2 µL of DNA per reaction and 40 cycles of PCR. The assembly of the nine strains shows that four fungal genotypes (*A. fumigatus*, *A. terreus*, *T. longibrachiatum* and *T. porphyrosporus*) were detected at 100 % nucleotide similarity: Contig 1 made up of the strains: TAM5, TAM6, TAM1, TAM4 belong to *T. longibrachiatum*; while Contig 2: TAM3, TAM7 corresponds to *A. fumigatus* TAM2 to *A. terreus*; and TAM9 to *T. porphyrosporus*.

All consensus sequences from these assemblies (Contigs) are greater than 96 % base quality (HQ %). Blast analyzes of the consensus sequence of Contig 1 (overlapping DNA segments) against GenBank and ISHAM suggest that this TAM5, TAM6, TAM1, TAM4 genotype corresponds to the species *T. longibrachiatum*. Blast analyzes of the consensus sequence of Contig 2 against GenBank and ISHAM suggest that this TAM3, TAM7 genotype corresponds to the species *A. fumigatus*. Blast analyzes of the consensus sequence of strain TAM2 against Gen

GenBank and ISHAM suggest that this genotype corresponds to the species *A. terreus*.

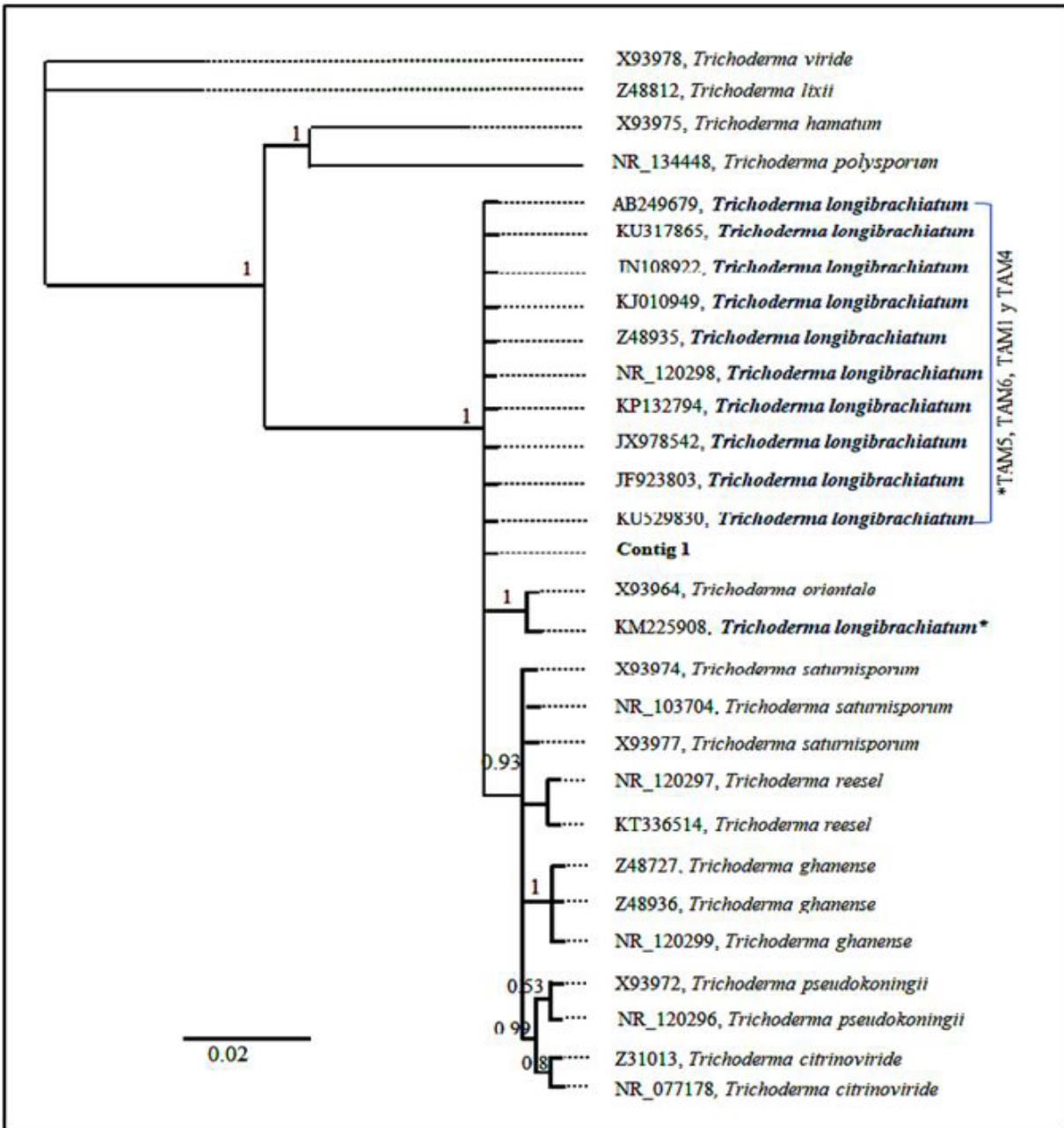
Blast analyzes of the consensus sequence of strain TAM9 against GenBank and UNITE suggest that this genotype corresponds to the species *T. porphyrosporus*. The identification of the *Trichoderma* genus was corroborated by phylogenetic analysis (Figure 2), which demonstrated that the Contig 1 genotype indeed belongs to the section *Longibrachiatum* with the highest level of support (Bpp = 1).

Within this section, Contig 1 is indeed found in the *T. longibrachiatum* clade with the highest level of support (Bpp = 1). Within this clade is the sequence of the type of strain (*T. longibrachiatum*) of this species with which Contig 1 has 100 % nucleotide similarity. Likewise, *Trichoderma* can produce enzymes and plant growth promoting compounds of biotechnological interest in agriculture. In the case of the *Aspergillus* genus, the phylogenetic analysis corroborates that Contig 2 indeed belongs to the *A. fumigatus* clade (Figure 3), with a high support value (Bpp = 0.89) and that the TAM2 strain indeed belongs to the A complex clade. *A. terreus* (Figure 4), with a high support value (Bpp = 0.81)

Kuhls *et al.* (1998), Guigón-López *et al.* (2010), Arrúa-Alvarenga *et al.* (2012) and Suárez-Contreras (2022), point out that for the identification of the genera of *Aspergillus* and *Trichoderma* at the species level, the ITS1 and ITS2 regions of the rDNA can be specifically used, in addition to the ITS4 region as was done in this study. However, although the identity levels are significant and the ITS region is considered a barcode for the taxonomic identification of fungi, the standard use of these ITS during PCR can generate biases, incurring error in the sequence, generating a trend in the classification of species within the divisions of the ascomycetes as noted by Menolli (2020).

**Figure 2**

Cladogram of *T. longibrachiatum* with the highest level of support ( $Bpp = 1$ )



**Note.** Within this clade is the sequence of the type of strain (NR\_120298) of this species with which Contig 1 has a 100 % nucleotide similarity.

Regarding the phylogenetic analysis that was carried out according to the Index Fungorum repository (<http://www.indexfungorum.org/names/Names.asp>), the strains identified in this study correspond to the phylum Ascomycota,

representing the subphyla Pezizomycotina and Saccharomycotina, clustered in the classes Eurotiomycetes associated with the orders Hypocreales and Eurotiales.



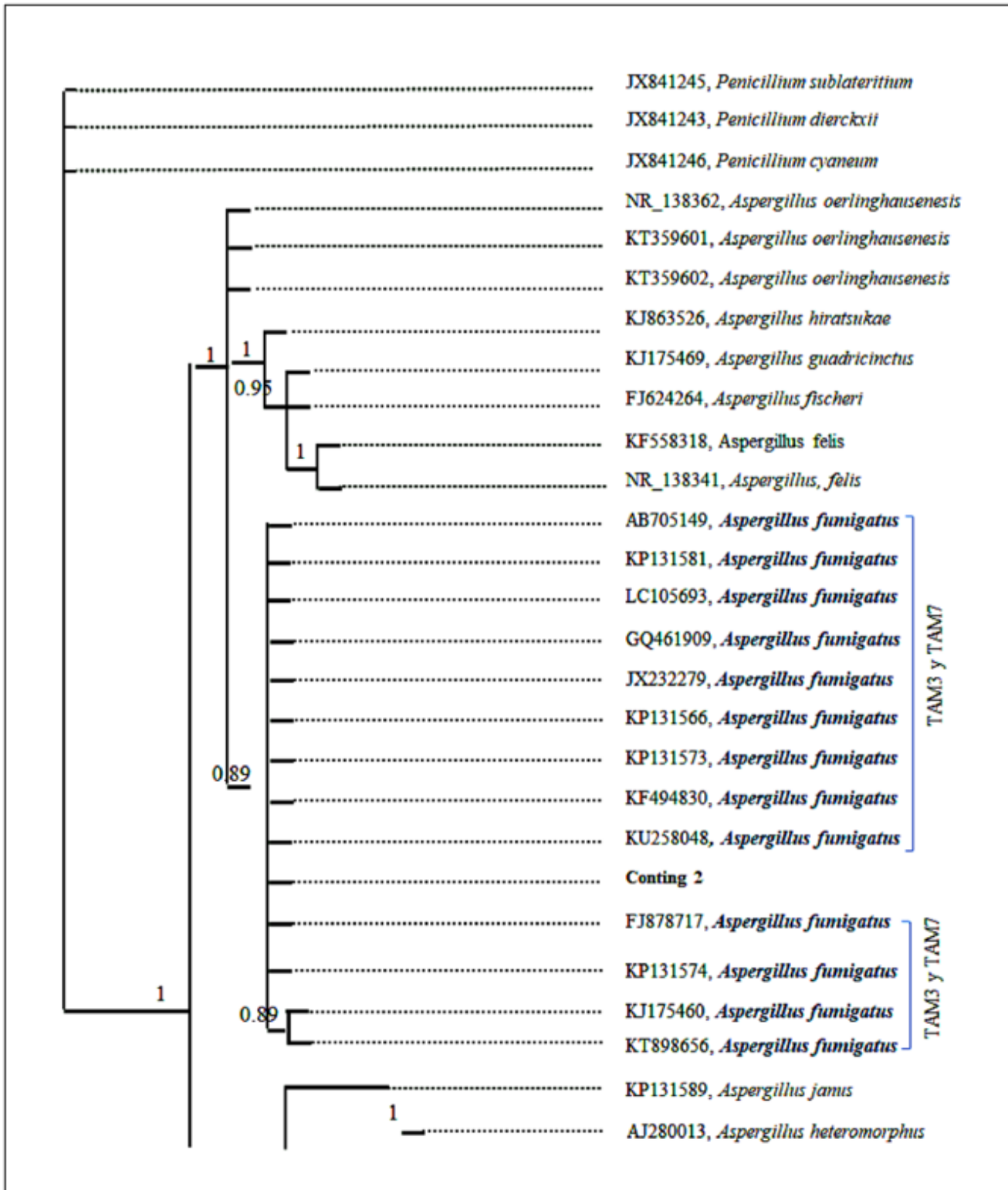
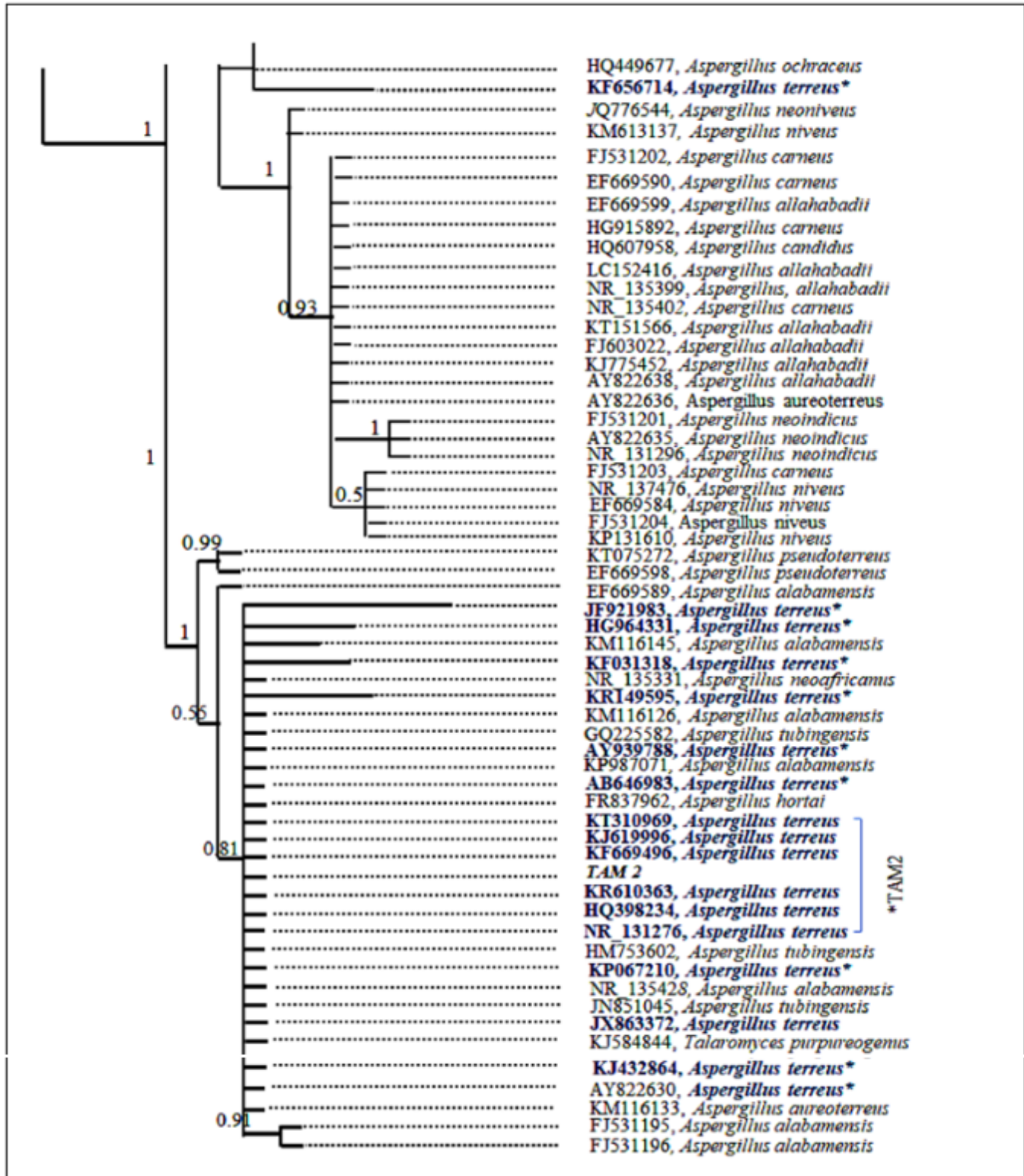


Figure 4

Cladogram of *Aspergillus terreus*. with the highest level of support (Bpp = 1)



In **Table 1**, only the TAM 9 strain was identified through nucleotide similarity because there are few sequences in the databases of this group, so phylogenetic analyzes cannot be performed. Among the similar sequences in Genbank are nine sequences that can be considered conspecific (nucleotide similarity

greater than 98.8 %) under the names *Tylopilus porphyrosporus*, *T. pseudoscaber*, *Porphyrellus pseudoscaber* and *P. porphyrosporus*. However, all of these are synonyms and the valid name according to Mycobank and the Index fungorum is *T. porphyrosporus*.

**Table 1**

*Significant alignment sequence*

Access number Genbank	Species	Longitud	Bits	Valor
JN021085	<i>Tylopilus porphyrosporus</i> (como <i>Porphyrellus</i> )	612	978	0.0
JF899578	<i>Tylopilus pseudoscaber</i>	813	978	0.0
EU685112	<i>Tylopilus porphyrosporus</i> (as <i>Porphyrellus</i> pse)	729	928	0.0
UDB024875	<i>Tylopilus pseudoscaber</i>   Canada	992	978	0.0
KC797154	<i>Tylopilus pseudoscaber</i>	659	964	0.0
UDB001485	<i>Tylopilus porphyrosporus</i> (como <i>Porphyrellus</i> )	685	964	0.0
KC152268	<i>Tylopilus porphyrosporus</i>	669	962	0.0
DQ534563	<i>Tylopilus porphyrosporus</i> (como <i>Porphyrellus</i> )	716	960	0.0
JF908788	<i>Tylopilus porphyrosporus</i>	484	843	0.0
KJ775786	<i>Tylopilus</i>	471	711	0.0
KJ775785	<i>Tylopilus pseudoscaber</i>	421	711	0.0
EU819450	<i>Tylopilus sordidus</i>	730	690	0.0
HE820600	Fungi	609	684	0.0
EU597078	<i>Tylopilus</i>	669	679	0.0
AB923009	Fungi	609	666	0.0
JQ991909	Boletaceae	Sin referencia	663	0.0
AB218092	Fungi	Sin referencia	657	0.0
KJ775787	<i>Tylopilus</i>	Sin referencia	646	0.0
UDB011971	<i>Tylopilus umbrosus</i>   Colombia	Sin referencia	637	0.0
UDB013038	Boletaceae   Papua New Guinea	Sin referencia	625	1,00E-177
AY970201	Basidiomycota	Sin referencia	609	8,00E-173
UDB013220	Boletaceae   Papua New Guinea	Sin referencia	609	8,00E-173
JF273512	Boletaceae	Sin referencia	598	1,00E-169
KM595001	Fungi	Sin referencia	544	3,00E-153
KM594960	Fungi	Sin referencia	544	3,00E-153
KM594937	Fungi	Sin referencia	544	3,00E-153
KM594899	Fungi	Sin referencia	544	3,00E-153
AB509640	Xerocomus	Sin referencia	461	3,00E-128
AB509756	Boletales	Sin referencia	459	9,00E-128
AB509993	Boletaceae	Sin referencia	452	1,00E-125
JN563923	Boletus	Sin referencia	385	2,00E-105
DQ679803	<i>Boletus pinophilus</i>	Sin referencia	378	2,00E-103
DQ131628	<i>Boletus pinophilus</i>	Sin referencia	378	2,00E-103
DQ131627	<i>Boletus pinophilus</i>	Sin referencia	378	2,00E-103
AY680973	<i>Boletus pinophilus</i>	Sin referencia	378	2,00E-103
AY680972	<i>Boletus pinophilus</i>	Sin referencia	378	2,00E-103
KC422606	<i>Boletus pinophilus</i>	Sin referencia	374	3,00E-102

## Biotechnological potential of the identified fungi

The potential of the fungi identified in this study is not only limited to their importance at the industrial level, but also to their value from the sanitary point of view, since many of them are direct and indirect pathogens that cause diseases in humans. Therefore, biotechnology plays an important role in the search for the transformation of these pathogenic fungi into fungi with industrial potential, such as *A. fumigatus*, *A. terreus* and *T. longibrachiatum*, so it is necessary to continue deepening the study of the identification and characterization of filamentous fungi, taking the studies to experimental phases. This way Qayyum *et al.* (2016) indicated that *A. terreus* is tolerant to concentrations of 50 mg of Cr present in sewage sludge. Murugaian *et al.* (2023), indicate that *A. terreus* has a greater tolerance and accumulation capacity for Cu. Medina *et al.* (2014), reported that *A. terreus* and *T. longibrachiatum* are capable of growing and degrading HTPs, observing a removal of over 85.00 % of these contaminants in *A. terreus*.

On the other hand, other authors have reported that *A. terreus* produces itaconic acid, an organic metabolite that is a material currently with high added value due to the presence of important properties that should replace different compounds that can be used in the manufacture of detergents, water treatments, dispersants, and adhesives (Li *et al.*, 2012).

*Aspergillus fumigatus* has biotechnological applications in the pharmaceutical industry (Mondal *et al.*, 2020). In addition to producing hydrolytic enzymes that have demonstrated biotechnological potential in the agricultural or agro-industrial sector. On the other hand, *T. longibrachiatum* produces enzymes that degrade lignocellulosic substrates; therefore, they are useful in the textile industry, which is why several authors have carried out their immobilization

for use in water treatment due to the discharge of dyes from this industry (Bagewadi *et al.*, 2017). This species has been observed to induce systemic resistance and expression of defense genes to prevent infection of pathogenic fungi such as *Botrytis cinerea* through activation of the synthesis of jasmonic acid, salicylic acid and ethylene (Yuan *et al.*, 2019) and inhibition of *Colletotrichum gloeosporioides*. In addition to its use in bioindustry (Andrade-Hoyosa *et al.*, 2023).

The *Trichoderma* genus is important in the environmental sector for its ability to bioremediate heavy metals in the sludge generated from wastewater treatment plants. This decontaminated sludge can be used as quality improvers and nutrient supplies in agricultural soils, in addition to produce enzymes and plant growth promoting compounds of biotechnological interest in agriculture (Hernández *et al.*, 2019). Finally, this study confirms the need to isolate and identify filamentous fungi from sewage sludge from wastewater treatment plants.

## Conclusions

Knowledge of the characteristics and mechanisms of filamentous fungi, in addition to their molecular and morphological characterization, will contribute to the application of biotechnological packages in the agricultural and environmental sectors. Future research should be carried out which should focus on evaluating the bioremediation potential of the species found in the residual sludge of the wastewater treatment plant, as well as determining which species is the most efficient in the removal of EPT in various contaminated sites. *Trichoderma longibrachiatum*, *Aspergillus terreus*, and *Aspergillus fumigatus* are the first report of species found in residual sludge from wastewater treatment plants in Mexico. *Tylopilus porphyrosporus* has not been reported in sewage or sewage sludge worldwide.

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